DOCKET NO.:PHOE-0061 **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Charles Mark Ensor, *et al.*

Serial No.: **09/921,380**

Group Art Unit: **1652**

Filing Date: **August 2, 2001**

Examiner: **Charles L. Patterson**

#9
B.G.J
8/14/03

For: **PEG-MODIFIED URICASE**

DECLARATION OF MIKE A. CLARK

I, Mike A. Clark, hereby declare that:

1) I am an inventor of the subject matter claimed in U.S. patent application Serial No. 09/921,380, filed August 2, 2001, and entitled "PEG-Modified Uricase."

2) I have reviewed the patent application (copy attached as Exhibit A), and I have also reviewed a set of patent claims (*i.e.*, claims 1 to 12, 21 to 25, 31 to 37, and 39 to 43, copy attached as Exhibit B) that I understand to be pending in the application.

3) The claimed subject matter provides, *inter alia*, compounds comprising uricase covalently bound via a linking group to polyethylene glycol, wherein the polyethylene glycol has a total weight average molecular weight of about 10,000 to about 30,000, and wherein the linking group is selected from the group consisting of a succinimide group, an amide group, an imide group, a carbamate group, an ester group, an epoxy group, a carboxyl group, a hydroxyl group, a carbohydrate, a tyrosine group, a cysteine group, a histidine group and combinations thereof.

4) Experiments were performed by myself or under my supervision in which purified *Candida utilis* uricase was covalently bound to polyethylene glycol (PEG) of various total

weight average molecular weights via various linking groups. The biological activity and the circulating half life of the various uricase preparations were determined.

5) In a particular set of experiments, purified *Candida utilis* uricase was covalently bound to polyethylene glycol of either a total weight average molecular weight of 5,000; 12,000; 20,000; 30,000; or 40,000 via a succinimidyl succinate linking group. Methoxy-succinimidyl succinate polyethylene glycol MW 5,000, methoxy-succinimidyl succinate polyethylene glycol MW 12,000, methoxy-succinimidyl succinate polyethylene glycol MW 20,000, methoxy-succinimidyl succinate polyethylene glycol MW 30,000, or methoxy-succinimidyl succinate polyethylene glycol MW 40,000 were separately added to solutions of uricase in 20 mM sodium phosphate buffer, pH 8,5 and the mixtures were stirred for 1 to 2 hours at room temperature. The solutions containing uricase conjugated to polyethylene glycol of the various molecular weights were then concentrated by diafiltration to approximately one-tenth of their original volume and were diafiltered against 10 volumes of a solution of 20 mM sodium phosphate buffer, pH 6.8 and 130 mM sodium chloride.

6) The enzymatic activity of the PEG-uricase preparations was determined using the uric acid diagnostic kit from Sigma (St. Louis, MO). Each PEG-uricase preparation was incubated with uric acid and the production of hydrogen peroxide was monitored by reaction with 4-aminoantipyrine and 3,5-dichloro-2-hydroxybenzenesulfonate. The activity of the PEG-uricase preparations was expressed as nmol hydrogen peroxide produced/min/mg of protein in the assay. The specific activity of uricase was expressed as IU/mL. One IU is defined as that amount of enzyme that produces 1 nmol of hydrogen peroxide/min

7) Uricase bound to polyethylene glycol of a weight average molecular weight of 5,000 retained 55% of the activity of native uricase. Uricase bound to polyethylene glycol of a weight average molecular weight of 12,000 retained 73% of the activity of native uricase.

Uricase bound to polyethylene glycol of a weight average molecular weight of 20,000 retained 75% of the activity of native uricase. Uricase bound to polyethylene glycol of a weight average molecular weight of 30,000 retained 74% of the activity of native uricase. Finally, uricase bound to polyethylene glycol of a weight average molecular weight of 40,000 retained 76% of the activity of native uricase. These results are presented in tabular form in Exhibit C.

8) The circulating half-life of native uricase and each of the PEG-uricase preparations was determined by injecting five mice with 1 IU of each uricase formulation. Blood samples were collected 1, 2, 3, 4, 8, 18, 24, 48, 72, and 96 hours later and each plasma sample was assayed *in vitro* for the amount of uricase present. The resulting data was plotted and the $t_{1/2}$ was determined as the mean of the data obtained from the five mice that received each uricase preparation.

9) The circulating half-life of native uricase was three hours. The circulating half-life of uricase bound to polyethylene glycol of a weight average molecular weight of 5,000 was 8 hours. The circulating half-life of uricase bound to polyethylene glycol of a weight average molecular weight of 12,000 was 24 hours. The circulating half-life of uricase bound to polyethylene glycol of a weight average molecular weight of 20,000 was 72 hours. The circulating half-life of uricase bound to polyethylene glycol of a weight average molecular weight of 30,000 was 84 hours. The circulating half-life of uricase bound to polyethylene glycol of a weight average molecular weight of 40,000 was 77 hours. These results are presented in tabular form in Exhibit C.

10) The results of these experiments indicate that purified *Candida utilis* uricase covalently bound to polyethylene glycol of weight average molecular weight of 12,000 to 40,000 retained approximately 75% of the biologically activity of native uricase. In addition,

as the molecular weight of the polyethylene glycol bound to uricase increased from 5,000 to 30,000, the circulating half-life of the PEG-uricase also increased from 8 to 84 hours.

11) I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

July 28 2003

Date

Mike A. Clark